REMARKS

I. Status of the Claims

Claims 62, 66, 75, 76, 79, and 81-83 are pending in the instant application. The rejection of claims 62, 66 and 81-83 (and now 75, 76 and 79) under 35 USC §112, second paragraph, has been maintained. The rejections of claims 62, 66 and 81-83 under 35 USC §102(b) or §103(a) over Shaw in view of Bazan, and claims 62, 66, 75, 76, 79 and 81-83 under 35 USC §103(a) over Shaw in view of Bazan and Bowie, have been maintained.

Claims 62, 66, 75, 76 and 79 are amended to delete reference to FIG. 4 and now make reference to FIG. 3. Inasmuch as each of the amended claims recited, prior to the amendment herein, G-CSF having the amino acid sequence set out in SEQ ID NO: 2, the legend to FIG. 3 expressly discloses the amino acid sequence of G-CSF being defined using the amino acid residue numbering as set out in SEQ ID NO: 2, and the legend to FIG. 4 also indicates that amino acids in G-CSF are defined as set out in SEQ ID NO: 2, the amendment includes no new matter.

II. The rejection of claims 62, 66, 75, 76, 79 and 81-83 under 35 USC §112, second paragraph, may be withdrawn.

Beginning at page 2 of the Office action, the Examiner maintained the rejection of claims 62, 66, 75, 76, 79 and 81-83 under 35 USC §112, second paragraph, for assertedly being indefinite. Specifically, the Examiner asserted that it is not clear which residues form the referenced external loops and further asserted that the application gives conflicting information as to the residues that form the loops between the helices thereby rendering the claims indefinite. Further, in view of the arguments presented in Applicant's amendment of March 23, 2007, the Examiner further asserted that different parties would come to different conclusions as to what modifications would and would not fall within the scope of the claims.

Notwithstanding Applicant's disagreement with the Examiner's assertions as articulated in Applicant's previous response, claims 62, 66, 75, 76, 79 (and thus 81-83) have been amended to recite "FIG. 3" rather than "FIG. 4." Applicant points out that the figure legend for FIG. 3 on page 25 of the specification as originally filed states the following:

> FIG. 3 is an "ribbon diagram" of the three dimensional structure of G-CSF. Helix A is amino acid residues 11-39 (numbered according to FIG. 1, above Seq. ID. No. 2), helix B is amino acid residues 72-91, helix C is amino acid residues 100-123, and helix D is amino acid residues 143-173. The relatively short 3^{10} helix is at amino acid residues 45-48, and the alpha helix is at amino acid residues 48-53. Residues 93-95 form almost one turn of a left handed helix.

In view of the legend for FIG. 3 which makes reference to SEQ ID NO: 2, the amendment to the claims as indicated above makes clear where the loops and helices of G-CSF, as set out in SEQ ID NO: 2 (recited in each of the claims prior to amendment), are located. Thus, in view of the reference to FIG. 3 and the teachings throughout the specification as filed, there can be no question as to what Applicant regards as an external loop as recited in the claims.

In view of the above, the rejection of claims 62, 66, 75, 76, 79 and 81-83 under 35 USC §112, second paragraph, may be withdrawn.

III. The rejection of claims 62, 66 and 81-83 under 35 USC §103(a), may be withdrawn.

Beginning at page 4 of the Office action, the Examiner maintained the rejection claims 62, 66 and 81-83 under 35 USC §103(a) as being directed to subject matter assertedly rendered obvious in view of the disclosure of Shaw (U.S. 4,904,584, hereinafter "Shaw"), and further in view of the disclosure of Bazan (Immunology Today, 11:350-54, hereinafter "Bazan"). After considering the arguments presented in Applicant's amendment of March 23, 2007, the Examiner asserted that the motivation to combine Shaw and Bazan is found in Shaw which teaches that those of ordinary skill in the art should look to the art for guidance as to what residues in G-CSF would be suitable targets for modification. The Examiner further asserted that although the teachings in Bazan and the instant application are not identical, those of ordinary skill in the art would have had a reasonable expectation of success in the combination of Shaw and Bazan for the making of operable PEGylated G-CSF variants.

Applicant respectfully submits that the Examiner's reliance on Bazan is an overstatement of what Bazan actually teaches. For example, at the bottom of page 4 of the Office action the Examiner stated:

> Nonetheless, the teachings of the reference indicate that the loop regions in general are appropriate targets for lysine modification, and provide sufficient information such that those of ordinary skill in the art would have a reasonable expectation of success in modifying the loop regions such that PEG molecules could be attached to lysines substituted therein.

The Examiner reaches this conclusion with an interpretation of Bazan as set out at page 5 of the Office action, wherein the Examiner further asserts,

> Moreover, while the Applicant points to specific guidance in the present application as to binding regions in the G-CSF molecule, it is noted that these regions generally conform to the teachings of the [*sic*] Bazan in that the binding regions are found in the helices.

This comment at page 5 of the Office action is not the first instance in which the Examiner interpreted Bazan this way. At page 9 in the Office action dated October 17, 2006, the Examiner stated,

> Additionally, the text on page 352 [of Bazan] indicates that G-CSF and homologous cytokines have their active regions (receptor binding regions) in the helical domains of the protein.

In both of these latter two statements, the Examiner implicated all G-CSF helices, or helical domains, as being required for receptor binding without qualifying these statements with the explicit disclosure of Bazan. By omitting the qualified disclosure of Bazan regarding G-CSF helix participation in receptor binding, the Examiner was able to construct the conclusion set out at page 4 of the Office action set out above.

Applicant respectfully traverses.

A. The Bazan reference does not teach that modifications should be made in loops.

Although the Examiner asserted that Bazan teaches the G-CSF loop regions are appropriate targets for lysine modification, the Examiner has not pointed to a single sentence in Bazan that evinces this position. Moreover, the Examiner has not pointed to any specific teaching in Bazan demonstrating that any G-CSF receptor binding site is located in any G-CSF helix region. The reason that the Examiner cannot point to these specific disclosures is because Bazan does not provide *any direct evidence* that receptor binding sites on G-CSF have been identified.

In terms of explicit teachings, Bazan at page 352 points to previous work by others that suggests for growth hormone releasing hormone (GRH), certain amino acid residues in helix D, the C-D loop and helix A are involved in receptor binding. Then *by analogy,* Bazan extrapolates these findings to apply to other cytokine thought to have similar overall structure:

> A strong clue to the existence of receptor-binding structural code is revealed by an elegant set of mutagenic experiments by Cunningham and Wells[53] that focus attention to an extended receptor-binding epitope in GRH formed by the exposed surface of helix D (with some adjoining residues from the nearby C-D loop and helix A; Fig. 2(b)). The analogous carboxy-terminal regions of PRL EPO, IL-6, MGF and G-CSF (Fig.2(a)) display a surprising match of periodically exposed residues, a similarity that centers on a conserved Phe/Tyr-Leu pair (Fig. 3(a)). The clear implication for this subset of cytokines is that the surface of predicted helix D is the primary receptor binding structure.

A mere extrapolation of analogous regions of one cytokine to another thought to be structurally similar without more does not provide a person of ordinary skill a reasonable expectation of success. That said, even if this disclosure is given its broadest interpretation and then applied to G-CSF, at most one *might* conclude that amino acids located in G-CSF helix D, helix A and the C-D loop are involved in receptor binding, and therefore, if modifications are to be made in G-CSF with the intent to retain receptor binding activity, one *might* conclude that it is best to introduce these changes in either the amino terminal region, the A-B loop, helix B, the B-C loop, helix C or the carboxy terminal region. As indicated below in more detail this conclusion would be error. Making one or more modifications to the A-B loop could result in disrupting the receptor binding activity as taught for the first time by the present inventor.

Adopting this broad interpretation, however, requires one to overlook what Bazan is actually stating. The section in Bazan quoted above from page 352 states "receptor-binding epitope in GRH formed by the exposed surface of helix D (with some adjoining residues from the nearby C-D loop and helix A; Fig. 2(b)." The clear statement is that <u>not all of the GRH helices</u> are necessary for receptor binding, and that at least a portion of one of the loops may be required for binding to the receptor. Second, to the extent that only certain exposed residues within helix D may be required for GRH binding, Bazan does not identify

which of these exposed residues may actually be required. One can reasonably conclude from this disclosure that Bazan is not suggesting that all residues in the helices are involved in receptor binding.

Thus, contrary to the Examiner's assertion, Bazan does not suggest "that the [receptor] binding regions are found in the helices" to a degree that no amino acid modifications can be made in any or all helix regions, and that any amino acid changes introduced with the expectation of retaining biological activity should be introduced into loop regions. Instead, Bazan *only* suggests that in GRH, some unidentified C-D loop residues, some unidentified A helix residues and the exposed D helix residues should be avoided as targets for amino acid modification. Moreover, to the extent that information regarding GRH can be extrapolated to other cytokines thought to be structurally related, Bazan cautions at page 353, left-hand column,

> The (convergent) similarity of certain key cytokine residues within the aligned recognition helices of Fig. 3(b) **suggests** a parallel similarity of binding sites in the set of cognate receptors. Progressive replacement of the helix residues (for example in the conversion of PRL to GRH-like cytokine) **may** probe individual amino acid contributions to specific binding. (Emphasis added.)

In other words, Bazan admits that knowledge about cytokines thought to be related to GRH is merely speculative, precluding any assertion by the Examiner about any expectation of success (much less a reasonable expectation) one might have before making amino acid changes in a G-CSF molecule. Indeed, until the present application was filed and the three-dimensional structure of G-CSF was made known for the first time, the speculation by Bazan remained exactly that, speculation.

In view of the above, the Examiner's contention that Bazan teach "the loop regions in general are appropriate targets for lysine modification" is conjecture that is not supported in Bazan.

B. Bazan fails to provide sufficient G-CSF structure-function information that would provide a person of ordinary skill in the art a reasonable expectation of success for generating biologically active G-CSF analogs.

The instant application is the first to demonstrate which domains in G-CSF are required for receptor binding. For example, beginning at page 69, line 19, in the present application, G-CSF receptor binding is described as follows:

> The domains required for G-CSF receptor binding were also determined based on the above analogs prepared and the G-CSF structure. The G-CSF receptor binding domain is located at residues (with methionine being position 1) 11-57 (between the A and AB helix) and 100-118 (between the B and C helices). One may also prepare abbreviated molecules capable of binding to a G-CSF receptor and initiate signal transduction for selectively stimulating neutrophils by changing the external loop structure and having the receptor binding domains remain intact.
>
> Residues essential for biological activity and presumably G-CSF receptor binding or signal transduction have been identified. Two distinct sites are located on two different regions of the secondary structure. What is here called "Site A" is located on a helix which is constrained by salt bridge contacts between two other members of the helical bundle. The second site, "Site B" is located on a relatively more flexible helix, AB. The AB helix is potentially more sensitive to local pH changes because of the type and position of the residues at the carboxy and amino termini. The functional importance of this flexible helix may be important in a conformationally induced fit when binding to the G-CSF receptor. Additionally, the extended portion of the D helix is also indicated to be a G-CSF receptor binding domain, as ascertained by direct mutational and indirect comparative protein structure analysis. Deletion of the carboxy terminal end of r-hu-met-G-CSF reduces activity as it does for hGH, see, Cunningham et al. Science 244: 1081-1084 (1989). Cytokines which have similar structures, such as IL-6 and GM-CSF with predicted similar topology also center their biological activity along the carboxy end of the D helix, see Bazan Immunology Today 11: 350-354 (1990). [Emphasis added]

The instant specification demonstrates the importance of these domains with a multitude of variants described throughout the examples.

From the above, it is clear that the binding domains found in G-CSF are in fact not identical to those Bazan describe for GRH. Without the G-CSF receptor binding domain information that was made available in the instant application (which is undeniably absent from the disclosure of Bazan), the worker of ordinary skill cannot predict involvement, or lack thereof, of any G-CSF loop amino acid residue in receptor binding from the Bazan

disclosure. Even assuming arguendo that Bazan teaches that loop residues should be modified, such a teaching could direct a person of ordinary skill in the art to modify residues between helix A and helix B which could, as taught for the first time in the instant specification, destroy biological activity. Bazan simply failed to appreciate the existence and therefore the role of the short A/B helix in G-CSF receptor binding.

Moreover, even if one accepted the prediction by Bazan that certain regions in the G-CSF structure were loops and other were helices, nothing in Bazan allows for prediction of the three dimensional model for the G-CSF molecule. Bazan merely predicted certain secondary structures in G-CSF based only on amino acid sequence similarity to other cytokines. Furthermore, simply identifying various secondary structures in a molecule does not identify which of these structures is exposed to the molecule's surface, or which are internalized and required for maintenance of a biologically active conformation. For example, a loop region may be buried between helices or sheets, instead of moving freely at the molecular surface, and in this buried location, the loop may interact with other secondary structures in a way that it helps maintain an overall active conformation. Because of these intricate interactions, the prediction of secondary structures based only on amino acid sequence extrapolations may not be particularly accurate.

Even though this possibility is not relevant to G-CSF as taught in the instant application, the discussion in the paragraph above demonstrates that simply identifying secondary structures by extrapolation does not account for all such structures that may be relevant for biological structure and function. For example, in the region Bazan discloses to be the A-B loop for G-CSF, the present applicant has identified a receptor binding site in the form of a previously unidentified helix (the AB helix), which, if modified (as the Examiner asserts that Bazan teaches), may result in loss of receptor binding. Unexpectedly superior properties, unexpectedly different properties, and the absence of expected properties are all relevant factors that can rebut a prima facie case of obviousness. In re Corkill, 711 F.2d 1496, 226103 USPQ 1005 (Fed. Cir. 1985)., In re Chupp, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987). The absence of a property which a claimed invention would have been expected to possess based on the teachings of the prior art is also evidence of unobviousness. See Ex parte Mead Johnson & Co. 227 USPQ 78 (Bd. Pat. App. & Inter. 1985).

Applicant submits that the identification of the AB helix (discussed further below) actually supports a finding of non-obviousness in view of the Examiner's assertion that "loop regions in general are appropriate targets for lysine modification, and provide sufficient information such that those of ordinary skill in the art would have a reasonable expectation of success in modifying the loop regions such that PEG molecules could be attached to lysines substituted therein."

C. The Examiner has overstated the teachings in Bazan to support assertions that are for the first time supported by the instant application

Considering the above, Applicant submits that the Examiner's over-reliance on Bazan is an attempt to shape the prior art into a teaching that is first disclosed in the instant specification. Such hindsight reconstruction that uses knowledge gleaned from Applicant's disclosure is forbidden[1]. As the Supreme Court noted in *KSR International v. Teleflex, Inc* 550 U.S. _____, (slip opinion at page 17), "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning. *See* [*Graham v. John Deere Co. of Kansas City*], 383 U. S., at 36 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to " 'guard against slipping into the use of hindsight' " (quoting *Monroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F. 2d 406, 412 (CA6 1964))).

Applicant notes that in prosecution of US Patent Application Serial No. 10/318,966 (Nissen et al.) (hereinafter the '966 application) which claims similar subject matter as the present application and is being examined by the same Examiner as in the instant application, a rejection under 35 USC §103 was also made which relied on all of the references cited in the instant rejection. However, in the '966 application, the Examiner further relied on US Patent 5,790,421 (hereinafter the '420 patent), in part, for the specific

[1] IN RE TRANSLOGIC TECHNOLOGY, INC. (2007 U.S. App. LEXIS 23969), a post-KSR case, states, in relevant part : "The Supreme Court observed that this court had also "elaborated a broader conception of the TSM test than was applied in [KSR]." Id. at 1743. Specifically the Court referred to Dystar Textilfarben GmbH & Co. v. C.H. Patrick Co., wherein [HN6] this court noted: "Our suggestion test is in actuality quite flexible and not only permits, but requires, consideration of common knowledge and common sense." 464 F.3d 1356, 1367 (Fed. Cir. 2006) (emphasis original). The Court suggested that this formulation would be more consistent with the Supreme Court's restatement of the TSM test. KSR Int'l Co., 127 S. Ct. at 1739. In any event, as the Supreme Court suggests, a flexible approach to the TSM test prevents hindsight and focuses on evidence before the time of invention, see, e.g., In re Rouffet, 149 F.3d 1350, 1357 (Fed. Cir. 1998), [*22] without unduly constraining the breadth of knowledge available to one of ordinary skill in the art during the obviousness analysis."

disclosure suggesting the loop regions as targets for attachment of PEG molecules. The specification of the '420 patent and the specification in the instant application are identical. Thus, it is evident that the Examiner is relying on the disclosure in the instant specification for its disclosure that G-CSF loops should be modified to support an obviousness rejection in the prosecution of Nissen et al. Applicant submits that while the rejection is proper in the Nissen et al., application in view of the disclosure in the '420 patent, there is no such art available to the Examiner which is material to claims in the instant application, and in the absence of available art which suggests modification of loop regions as claimed herein, the Examiner has developed by overstatement an interpretation of the Bazan disclosure that is simply not found in the reference. Were the Shaw and Bazan references relied upon for rejecting claims in this application sufficient, the Examiner would not have needed to add the disclosure of the '420 patent to reject similar subject matter in the Nissan '966 application

Considering all of the foregoing arguments, Applicant submits that the Examiner has overstated and shaped the teachings in Bazan to support assertions that are for the first time disclosed to the world by the instant application. Such an overstatement in support of the 35 USC §103 rejection of claims 62, 66 and 81-83 amounts to an impermissible use of hindsight using the Applicant's own disclosure and should be withdrawn

IV. The rejection of claims 62, 66, 75, 76, 79 and 81-83 under 35 USC §103(a), may be withdrawn.

Beginning at page 5 of the Office action, the Examiner maintained the rejections of claims 62, 66, 75, 76, 79 and 81-83 under 35 USC §103(a) as being obvious over Shaw, in view of Bazan and further in view of Bowie et al. After considering the arguments presented in Applicant's amendment of March 23, 2007, the Examiner asserted that Bowie teaches that those of ordinary skill in the art would have a reasonable expectation that modifications could be made to proteins without loss of function. The Examiner further asserted that the teachings in O'Shea (Science, 254:539-544) and Tsuji (Proteins, 9:12-22) further indicate that modifications in external helical regions could be used to stabilize proteins (and thus would be motivated to make such modifications). Finally, the Examiner asserted that the present claims do not identify a non-obvious variant of G-CSF since the present claims do not ascribe any particular function to the recited modifications and since

Bowie teaches that proteins are capable of tolerating modifications without loss of function. Applicant respectfully traverses.

At the outset, Applicant repeats the arguments set forth above with respect to Shaw and Bazan. Shaw fails to teach modifying G-CSF by substituting residues in loop regions, and Bazan does not remedy this failure nor does it not motivate the skilled worker to make changes in loop regions. Likewise, neither Bowie, O'Shea nor Tsuji rectify this deficiency. Indeed, Bowie, O'Shea and Tsuji are completely silent with regard to G-CSF, let alone identifying any specific regions in G-CSF and making modifications therein. As a result, the combined disclosures of Bowie, Shaw and Bazan cannot render obvious subject matter of claims 62, 66, 75, 76, 79 and 81-83. At best, as indicated above, the rejection under 35 USC §103(a) is the result of the use of impermissible hindsight using the Applicant's own disclosure and consequently must be withdrawn.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

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